



Tremella fuciformis polysaccharides as a fat substitute on the rheological, texture and sensory attributes of low-fat yogurt

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ARTICLE INFO

Handling Editor: Alejandro G. Marangoni

Keywords:

Tremella fuciformis polysaccharides
Water holding capacity
Fat substitute
Syneresis
Structure
Dairy products

ABSTRACT

The potential of *Tremella fuciformis* polysaccharides (TFPS) as a fat substitute in low-fat yogurt was evaluated in this study. The effects of adding different concentrations of TFPS solution on the physical and chemical properties, texture, rheology, microstructure and sensory properties of low-fat yogurt were evaluated. Compared with control, the addition of TFPS not only increased the solid content and water holding capacity of yogurt, but also reduced syneresis losses in low-fat yogurt. In fact, the addition of TFPS did not affect the color of yogurt but had a positive effect on the texture and sensory of yogurt. In terms of rheology, all low-yogurt samples exhibited rheological to the weak gel-like structures ($G' > G''$), and the storage modulus and loss modulus of the yogurt added with TFPS were higher than those of the low-fat yogurt control group. Compared with the low-fat yogurt control group, yogurt added TFPS makes the cross-linking of polysaccharides and casein more compact. In conclusion, TFPS has potential as a fat substitute in dairy products.

1. Introduction

Yogurt has long been considered a healthier drink, and is gradually becoming one of the favorite beverage of consumers due to its high nutrients content and beneficial effects on gut (Wang et al., 2022). As we all know, excessive fat accumulation in the body causes overweight and obesity that is associated with an increased risk of several chronic diseases (Zhang et al., 2021; Liu et al., 2022). To advocate a green and healthy life, realizing healthy and delicious beverages is a major pursuit of current consumers.

The texture and rheology of yogurt are key quality trait factors favored by consumers. Fat globules are the fundamental source of hardness and rheology (Santiago-García et al., 2021). Due to the cross-linking of fat globules and protein, the yogurt has a silky and full-bodied taste. For dietary nutrition and good health, consumers consciously reduce fat intake, and low-fat yogurt came into being, which has broad market prospects. According to the Food and Drug Administration (FDA) US federal regulations, the fat content of low-fat yogurt must not exceed 2.0% (Khubber et al., 2021). The fat content is vital factor which determine the sensory and quality of yogurt (Mary, Muturi, & Kapoor, 2022). The reduction of fat content in yogurt reduces the solid content and affects the flavor and structure of the yogurt, which

ultimately leads to low viscosity and poor taste. Therefore, finding suitable fat substitutes to meet consumers' health needs is challenging.

The application of fat substitutes in yogurt has emerged in the recent years. Carbohydrate was one of the most common fat substitutes, especially polysaccharides, such as inulin and okra polysaccharides (Yang et al., 2020). Studies have shown that the addition of polysaccharides can improve whey precipitation and poor taste of low fat yogurt (Zhao et al., 2020). These carbohydrates were classified as dietary fiber with prebiotic ability, which can improve the texture and structure of low-fat yogurt (Santiago-García et al., 2021). Inulin-type fructans have been widely used as fat substitutes in low-fat dairy products and can form microcrystals when mixed with water or milk (Teferra, 2021). Not only are these crystals not found in the oral cavity, but also its interacted with the food matrix to form a delicate creamy texture, producing a feeling similar to whole milk (Arango et al., 2020). Similarly, the addition of polysaccharide such as gum arabic, gelatin and pectin to yogurt can maintain dimensional stability and change the rheology and texture properties of yogurt through the negatively charged carboxyl groups and positively charged groups (Huang et al., 2021; Wusigale Liang and Luo, 2020).

Polysaccharide was the main active ingredient of *Tremella* extract, which can be used as a gelling agent and thickening agent for foods

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<https://doi.org/10.1016/j.crfs.2022.06.007>

Received 3 April 2022; Received in revised form 2 June 2022; Accepted 16 June 2022

Available online 22 June 2022

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(Sheng et al., 2021). In addition to forming a high-viscosity solution in an aqueous solution to produce fat rheological properties, it can also be used to simulate fat (Zhang et al., 2017). As a good source of prebiotics (Liu et al., 2020), TFPS have a synergistic effect with the nutritional and therapeutic properties of yogurt to produce beneficial health effects (Yang et al., 2020).

Currently, there is very limited information on the use of polysaccharides to replace fat in dairy products. This study aims to explore the feasibility of TFPS in low-fat yogurt to replace fat. The effects of TFPS on the physical and chemical properties, texture, rheology and microstructure of low-fat yogurt were studied.

2. Materials and methods

2.1. Materials

Whole milk (fat 3.0 g/100 mL, protein 3.8 g/100 mL) and low-fat milk (fat 1.5 g/100 mL, protein 3.8 g/100 mL) were obtained from Hangzhou New Hope Shuangfeng Dairy Co., Ltd. TFPS powder was purchased from Hangzhou Johncan Biotech Co, Ltd. (Hangzhou, China). Starter culture contains *Bacillus bulgaricus* and *Streptococcus thermophilus* (Yo-Mix 387 LYO) used for the fermentation of yogurt was supplied by Danisco Co. Ltd. (Shanghai, China). Sodium hydroxide (96%), phenolphthalein (98%), sodium tetraborate (99.5%), sodium lauryl sulfate (99%), o-phthalaldehyde (97%) and dimercaptotrehitol (99%) were all analytical grades.

2.2. Yogurt manufacture

The samples were prepared with different concentrations of TFPS and were designated as follows: 3.0%FFY (yogurt made from full-fat milk: a control group), LY Control (made with low-fat milk: a control group), 0.025%TLY (0.025%TFPS added to low-fat yogurt), 0.050%TLY (0.050%TFPS added to low-fat yogurt), 0.075%TLY (0.075%TFPS added to low-fat yogurt), 0.100%TLY (0.100%TFPS added to low-fat yogurt). First, ensure the homogeneity of the solution by dissolving the TFPS in water with continuously stirring using a magnetic stirrer. Subsequently, the homogenized solution was hydrated at 4 °C for 48 h. Then, put the low-fat milk, sucrose (7 g/100 mL), and TFPS solution into a pre-sterilized beaker in a certain proportion. The solution was mixed and stirred, heated to 65 °C, and homogenized under the condition of 18 Mpa with a high-pressure homogenizer (model APV 1000, APV Systems, Beijing, China). The homogenized solution was pasteurized at 95 °C for 5 min and cooled to 42 °C in a cold-water bath during inoculation. Then add the bacteria in the ratio of 1:1000 (bacteria solution: solution) and stir evenly. For fermentation, the sample was placed in a 38 °C incubator to ferment for 4–5 h until the pH of the yogurt reaches 4.6. Finally, the yogurt samples were refrigerated (4 ± 1 °C) for 21 days for analysis.

2.3. Determination of the physical and chemical properties of yogurt

The physicochemical properties of TFPS yogurts were analyzed at 1, 7, 14 and 21 days of storage at 4 ± 1 °C. At each sampling day, physicochemical analysis (using triplicate samples) was carried out.

2.3.1. pH and titratable acidity (TTA) analysis

The pH of samples was determined by a pH-meter at room temperature (PHSJ-5; INESA; Shanghai). The titration method of AOAC (1990) was used to measure TTA of yogurts, and its value was expressed as °D. Briefly, a sample dissolved in distilled water was titrated to slightly pink and lasts up to 30 s without fading, using the phenolphthalein as an indicator (Parvaree et al., 2021).

2.3.2. Total solids content

According to the method described by Xu et al. (2019), the total solid content of the yogurt was analyzed by drying the yogurt in an oven at

100 ± 2 °C. The refractometer (Boeco Digital Abbe Refractometer, BOE 32400, Germany) was used to determine the content of soluble solids. Briefly, two to three drops of well-stirred yogurt re-titrated on a calibrated refractometer at 20 °C, then the prism was quickly closed, and the value was recorded.

2.3.3. Color

The color properties of yogurts during storage (4 °C) were measured with colorimeter (CR-400, Konica Minolta, Osaka, Japan) which was first calibrated with a blackboard and a whiteboard, according to the method of Pan et al. (2019). The color properties of yogurts were exhibited as L* (brightness, white = 100, black = 0), a* (positive values mean red; negative values mean green), and b* (positive values mean yellow; negative values mean blue).

2.3.4. Water holding capacity (WHC)

Determination of yogurt samples were carried out using the method of Parvaree et al. (2021). Samples were centrifuged at 2000 r/min for 20 min at 4 °C. WHC was calculated by the following formula:

$$WHC (\%) = (W_3 - W_1) / (W_2 - W_1) \times 100 \quad (1)$$

where W_1 is the weight of the centrifuge tube, W_2 is the weight of yogurt and centrifuge tube, W_3 is the weight of the upper liquid absorbed after centrifugation.

2.3.5. Syneresis

The syneresis is determined by gravity separation of milk proteins and polysaccharides, which is one of the indicators reflecting the quality of yogurt. Syneresis of yogurt samples was measured after 21 days of refrigeration according with the protocol described by Kim et al. (2020). The Syneresis was calculated by the following formula:

$$Syneresis (\%) = [\text{separated whey (g)}] / [\text{sample weight (g)}] \times 100 \quad (2)$$

2.3.6. Extent of proteolysis measurement

Filtrates of the yogurt samples were determined according to the methods of Ramchandran and Shah (2010). First, the yogurt sample was centrifuged at 4000 × g for 30 min at 4 °C. The supernatant was collected and filtered with a 0.45-µm membrane filter and stored at -20 °C until analysis. The degree of decomposition of free amino acids in the yogurt filtrate was determined by methods of Dai et al. (2018). Briefly, 50 µL of filtrate was added to the o-phthalaldehyde solution, shaken, and perform the measurement with a microplate reader (Glo-Max Discover Multimode Microplate Reader, Promega, Madison, WI, USA) under the condition of 340 nm within 2 min. Extent of proteolysis was judged based on the value of absorbance at 340 nm.

2.4. Determination of the quality characteristics of yogurt

The quality characteristics of TFPS yogurts were analyzed at 1 day and after 7, 14 and 21 days of storage at 4 ± 2 °C. On each sampling day, quality analysis (using triplicate samples) was carried out.

2.4.1. Texture Profile Analysis (TPA)

All samples were performed in triplicate using a TA. XT Plus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a p/100 probe (100 mm) with slight modification (Zhao et al., 2020). The test was completed in a petri dish with a diameter of 12 cm. The probe was set with pre-test speed = 1 mm/s, test speed = 2 mm/s, post-test speed = 10 mm/s and trigger force = auto.

2.4.2. Rheological measurements

Physical MCR 52 rheometer system (Anton Pa Aar GmbH, Austria) with a plate geometry sensor (PP-50, 1.000 mm gap) were used to detect the rheological properties of yogurt samples. At the pretest sessions, the sample was stirred and left at 4 °C for 5 min. After the rheometer was

calibrated, the yogurt sample was transferred to the rheometer plate, and the upper parallel plate was adjusted. Excess sample was then removed, and the measurement of rotating shear and oscillatory shear were performed.

In the rotating shear mode, the flow curve connected by 33 points were recorded with a logarithmic sweep shear rate from 0.01 to 100 s⁻¹. The frequency sweep test was performed in the range of 0.1–100 rad⁻¹ at a strain of 0.1% at 25 °C (Santiago-García et al., 2021). The experimental data was collected using the software provided by the manufacturer. The storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) were automatically obtained during each run. Through the strain sweep experiment, it is confirmed that all the measurements were in the experimental linear viscoelastic elasticity within the scope.

2.5. Microstructure of the yogurts by scanning electron microscope (SEM)

The microscopic morphology of the yogurt stored at 4 °C on the 1st and 21st day of storage was observed by scanning electron microscope (Sigma 300 VP, Zeiss, Oberkochen, Germany), according to the method

described by Benmeziene et al. (2021) with few modifications. The tested yogurt samples were yogurt stored at 4 °C. The yogurts samples were freeze-dried for 48 h, and gold sputtered in the vacuum coating unit for 1 min. The electron acceleration voltage was 5 kV, and different magnifications (× 2500 and × 10000) were used to visualize the structure.

2.6. Sensory evaluation

Sensory analysis was evaluated based on previous consumer preferences (Sheng et al., 2021). The samples included whole yogurt and low-fat yogurt as controls, as well as yogurt containing different concentrations of TFPS. All samples were packed in transparent plastic cups and numbered randomly. The sensory characteristics of yogurt on the 1, 7, 14, and 21 days meeting the requirements of ISO8589 were evaluated. Water was available to remove residual flavor of the previous samples to prevent the taste evaluation from being affected. The evaluation was carried out by ten reviewers, and the selection of panel members based on their product experience and food background. Half of reviewers

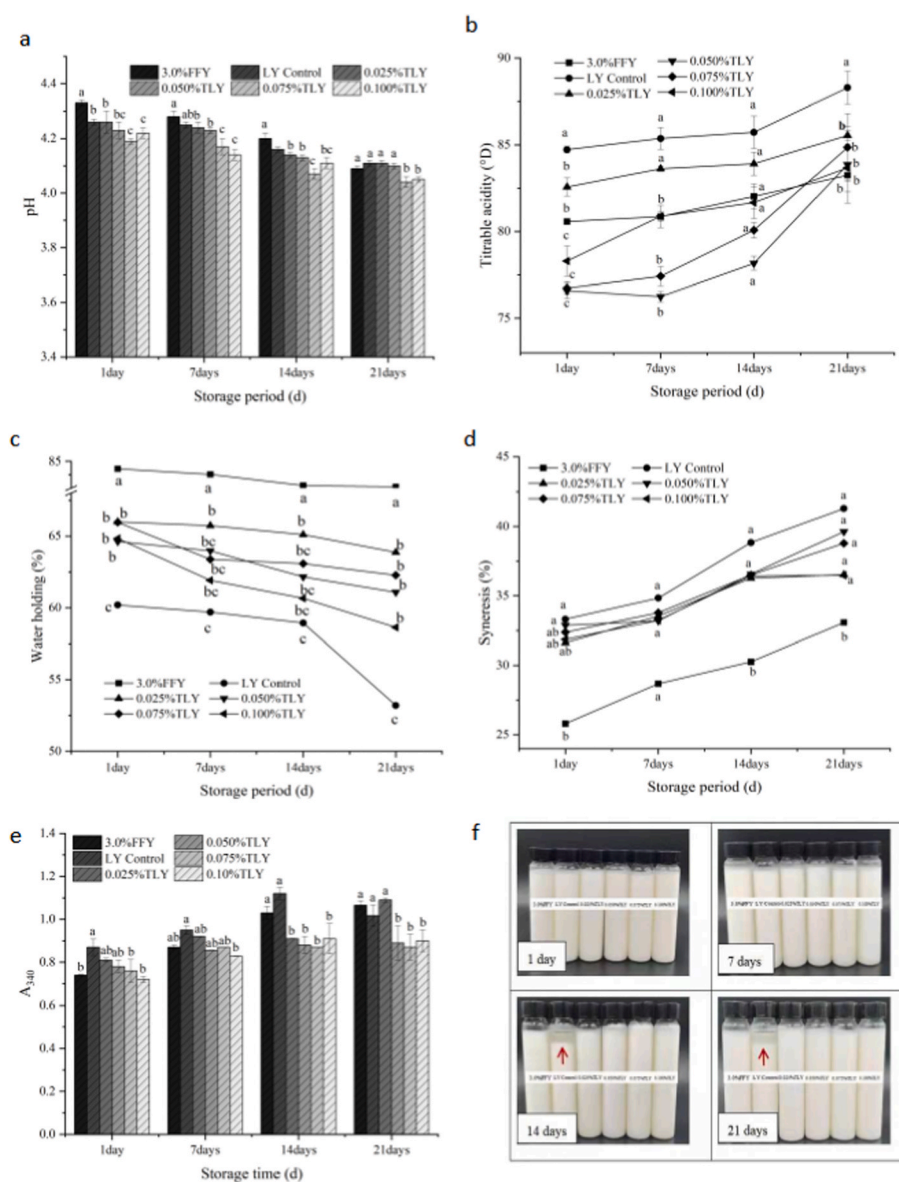


Fig. 1. The physical and chemical properties of different yogurt samples after storage for 21 days. (a: pH value; b: titratable acidity; c: water holding capacity; d: syneresis; e: proteolysis; f: Whey extraction). Means sharing the same letter do not differ significantly ($p \leq 0.05$). Different letters show statistically significant results ($p < 0.05$) of each sample during storage.

come from a dairy company, and the other half are students from the department of Food Science and Technology, Zhejiang University of Technology who have experience in sensory evaluation. The evaluation of yogurt samples included the following sensory attributes: appearance, color, texture, acidity, taste and overall acceptability. The scale used is the 9-point Hedonic scale, where 1 means dislike extremely, 5 means neither dislike nor like, and 9 means like extremely (Vénica et al., 2019).

2.7. Statistical analysis

Graph prism was used for statistical analysis. Values were represented as mean \pm standard deviation (SD). Statistical differences were determined by one-way ANOVA, followed by Tukey multiple comparisons test. The average value was compared with the least significant difference test when the $p \leq 0.05$, which was suitable for yogurt with different storage time. All figures are drawn by origin 9.0 (Origin Lab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Physical and chemical properties of yogurt samples

3.1.1. pH, TTA, total solids and soluble solids content

The pH value affects the texture and taste of yogurt (Damin et al., 2009). The effects of different concentrations of TFPS on the pH of yogurt was presented in Fig. 1(a). The results showed that with the increase of the concentration of TFPS, the pH of 3.0% FFY yogurt was 4.33 after incubation and reduced to 4.09 after day 21 at 4 °C. On the one hand, due to the hydrolysis of lactose, the bacteria have a high metabolic activity, which results in an increase in the content of lactic acid (Aoi et al., 2020). On the other hand, the addition of polysaccharides increases the number of microorganisms in yogurt, promotes the fermentation of lactic acid bacteria, and accelerates the accumulation of organic acids, thereby reducing the pH (Zhao et al., 2020).

Over acidification of yogurt during storage is an undesirable feature of yogurt (Deshwal et al., 2021). On the 7th and 14th days, there was no difference in TTA between 3.0% FFY yogurt and yogurt added with TFPS (Fig. 1(b)), indicating that the TTA of yogurt has independent of the amount of fat content. In the 0.005% TLY and 0.075% TLY groups, within 14–21 days, the TTA significantly increased, which was likely due to the further acid-forming activity of yogurt bacteria during storage (Nguyen et al., 2018). This result of increased TTA indicated the prebiotic effect of TFPS on yogurt culture (Shanmugam et al., 2022). Overall, within 21 days, the pH value showed a downward trend, and the TTA ranged from 75 °D to 90 °D. However, the addition of TFPS had no effect on the overall trend of pH and TTA in yogurt.

Table 1

Change in color value and Total solids of yogurt during 21 days of storage.

Storage time		3.0% FFY	LY Control	0.025% TLY	0.050% TLY	0.075% TLY	0.100% TLY
1 day	L*	91.03 \pm 2.43 ^a	90.67 \pm 0.50 ^{ab}	90.48 \pm 0.45 ^a	88.50 \pm 1.20 ^b	89.38 \pm 0.41 ^b	88.44 \pm 0.50 ^b
	a*	-0.68 \pm 0.14 ^a	-1.44 \pm 0.01 ^a	-1.38 \pm 0.07 ^a	-1.24 \pm 0.10 ^a	-1.47 \pm 0.07 ^a	-1.40 \pm 0.07 ^a
	b*	3.63 \pm 0.84 ^a	3.15 \pm 0.27 ^a	3.21 \pm 0.19 ^a	3.15 \pm 0.28 ^a	2.95 \pm 0.41 ^a	3.35 \pm 0.16 ^a
	Total solids (%)	18.06 \pm 0.01 ^a	15.27 \pm 0.02 ^c	15.30 \pm 0.01 ^c	16.26 \pm 0.07 ^{bc}	16.44 \pm 0.01 ^b	16.54 \pm 0.01 ^c
7 days	L*	81.03 \pm 2.47 ^a	78.96 \pm 2.18 ^{ab}	79.93 \pm 1.38 ^a	79.43 \pm 0.06 ^b	79.42 \pm 0.36 ^b	79.72 \pm 1.50 ^c
	a*	-0.60 \pm 0.02 ^a	-0.84 \pm 0.06 ^a	-1.06 \pm 0.06 ^a	-1.43 \pm 0.04 ^a	-1.40 \pm 0.09 ^a	-1.03 \pm 0.06 ^a
	b*	3.08 \pm 0.33 ^a	1.81 \pm 0.33 ^a	2.22 \pm 0.27 ^a	2.28 \pm 0.23 ^a	1.99 \pm 0.34 ^a	2.41 \pm 0.13 ^a
	Total solids (%)	17.97 \pm 0.05 ^a	15.49 \pm 0.01 ^c	16.36 \pm 0.02 ^{bc}	16.57 \pm 0.01 ^b	16.59 \pm 0.02 ^b	15.49 \pm 0.01 ^c
14 days	L*	75.69 \pm 1.05 ^{bc}	77.92 \pm 0.97 ^b	79.19 \pm 1.01 ^c	76.41 \pm 1.32 ^c	76.02 \pm 1.87 ^c	73.67 \pm 1.52 ^c
	a*	-1.59 \pm 0.16 ^a	-1.15 \pm 0.10 ^a	-1.21 \pm 0.18 ^a	-1.28 \pm 0.16 ^a	-1.12 \pm 0.08 ^a	-1.10 \pm 0.08 ^a
	b*	3.20 \pm 0.28 ^a	2.64 \pm 0.24 ^a	2.69 \pm 0.37 ^a	2.59 \pm 0.17 ^a	3.10 \pm 0.23 ^a	2.68 \pm 0.32 ^a
	Total solids (%)	17.12 \pm 0.01 ^{bd}	15.22 \pm 0.03 ^{ce}	16.24 \pm 0.01 ^{ade}	16.44 \pm 0.02 ^{ad}	16.54 \pm 0.01 ^d	15.35 \pm 0.01 ^e
21 days	L*	73.20 \pm 2.27 ^a	77.84 \pm 2.12 ^{ac}	82.21 \pm 0.29 ^b	76.10 \pm 1.38 ^c	75.49 \pm 0.91 ^c	72.71 \pm 1.30 ^d
	a*	-1.74 \pm 0.09 ^a	-0.84 \pm 0.08 ^a	-1.32 \pm 0.03 ^a	-1.05 \pm 0.03 ^a	-1.16 \pm 0.07 ^a	-1.04 \pm 0.07 ^a
	b*	3.33 \pm 0.24 ^a	2.11 \pm 0.14 ^a	2.08 \pm 0.05 ^a	2.19 \pm 0.65 ^a	2.28 \pm 0.25 ^a	2.22 \pm 0.30 ^a
	Total solids (%)	16.92 \pm 0.02 ^a	15.11 \pm 0.01 ^c	16.13 \pm 0.02 ^{bc}	16.29 \pm 0.01 ^b	16.46 \pm 0.01 ^b	15.29 \pm 0.02 ^c

Means in the same column shown with different letters are significantly different ($p < 0.05$).

The increase in solid content depends on the additives in yogurt (Ujiroghene et al., 2019). Similarly, total solids and soluble solids content increased with the higher concentration of TFPS in this study. However, with the prolongation of storage time, the total solids and soluble solids content gradually decreased. As a carbon source, part of polysaccharides were decomposed by lactic acid bacteria, which subsequently increasing the TTA of the yogurt (Kycia et al., 2020).

3.1.2. Color

Color is one of the most recognizable visual features in dairy products, and one of the parameters for consumers choosing food (Wang et al., 2022). The color properties of yogurt are shown in Table 1. The L* value of the 3.0% FFY and LY Control group was higher than the other four groups of yogurts, indicating that the addition of TFPS had a slight effect on the brightness of the yogurt. As the concentration of TFPS increased, the L* value decreased from 91.03 \pm 2.43 to 88.44 \pm 0.50, showing that correlation between the L* value and amounts of TFPS addition. The L* value of TFPS solution decreased with the addition of different concentrations and showed significant differences between different concentrations. Therefore, the color of TFPS itself affected the L* value of yogurt (Supplementary Table S1). There was no significant ($p > 0.05$) difference in the a* and b* values of yogurts with different concentration of TFPS. Similarly, Pan et al. (2019) also reported that similar quince seed mucilage powder added to yogurt has no significant difference in a* value and b* value by the storage time. All in all, the color of yogurt was not affected by the addition of TFPS.

3.1.3. WHC

From Fig. 1(c), the WHC of 3% FFY was as high as 83.65%, which was obviously higher than the WHC of other five low-fat yogurts groups. Reduced fat content leads to the weakening of the internal structure of the yogurt, resulting in the separation of whey (Dai et al., 2016). The WHC of the yogurt added with TFPS was greater than that of LY Control, reflecting the ability of TFPS to bind water. TFPS combined with proteins to form a three-dimensional network structure, with a compact structure and good water locking effect. The increase of concentration of TFPS have negative effect on the WHC of yogurts, which was due to the excessive polysaccharides destroyed the network structure. The higher concentration of Tremella polysaccharides was easy to form a hinge structure, which leads to the outflow of water and the decrease of WHC (Zhang et al., 2017).

3.1.4. Syneresis

As shown in Fig. 1(d), the syneresis trend was negatively correlated with the WHC trend in 3.1.3. The syneresis of whole yogurt was lowest, because the fat globules of whole milk increase the density of protein,

preventing the precipitation of whey (Gilbert et al., 2020). In the absence of fat or low fat content, the addition of polysaccharides can reduce the precipitation of whey (Nguyen et al., 2017), enhances the density of the gel network structure and reduces the loss of whey. The syneresis of LY Control was the highest, indicating that whey was easier to fall off from the gel network structure. Adding TFPS to yogurt, the whey precipitation of yogurts was slightly improved, among them, 0.025% TLY yogurt has the lowest syneresis of 31.63%. This finding was consistent with that of Jia et al. (2022) who found the addition of polysaccharides such as corn starch effectively reduced the syneresis of yogurts. Continuously decreasing pH value during storage leads to a slow increase in syneresis, favoring rearrangement of the gel network. Fig. 1(f) showed the whey precipitation for 21 days, of which LY Control has the most whey precipitation and less yogurt containing TFPS, which also showed that TFPS has a positive effect on the whey precipitation of yogurt.

3.1.5. Extent of proteolysis

The extent of proteolysis represents the protein hydrolysis ability of

bacteria in yogurt, and it is also closely related to the degree of acidification of yogurt (Ramchandran and Shah, 2010). As shown in Fig. 1(e), during the storage period, the protein hydrolysis of all yogurts increased, but the increase magnitude of different samples was different. The content of free amino acids rapidly increased in the first 14 days, and then leveled off after 14 days. The protease and peptidase of lactic acid bacteria can hydrolyze the protein in milk, increasing the content of free amino acids (Li and Shah, 2015). Lactic acid bacteria cannot fully utilize the amino acids and peptides released by casein at an early stage, leading to the accumulation of peptides and amino acids. The release and consumption of peptides tend to be balanced until 14–21 days. There was no significant difference of proteolysis extent between whole and low-fat yogurt, indicating that the degree of protein breakdown was irrelevant to the fat content. The free amino acid content of 0.050% TLY, 0.075% TLY and 0.100% TLY of the three yogurts was lower than 3.0% FFY, which indicated that polysaccharides and proteins form a tight network structure, reducing the extent of proteolysis (Nielsen et al., 2022). However, from Fig. 1(e), it can be seen that the absorbance difference of the proteolysis degree in six group yogurts in the in the

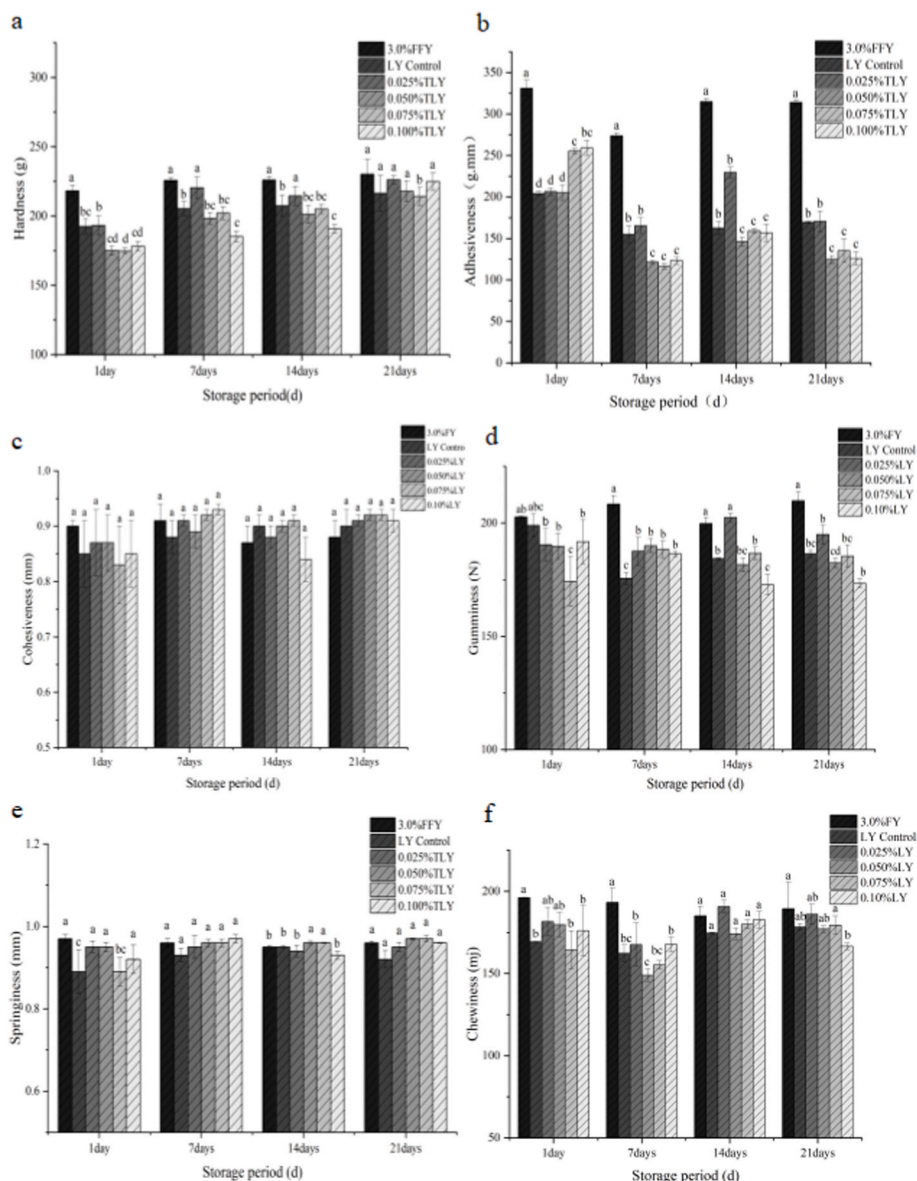


Fig. 2. The texture of different yogurt samples after storage for 21 days. (a: Hardness; b: Adhesiveness; c: Cohesiveness; d: Gumminess; e: Springiness; f: Chewiness). Means sharing the same letter do not differ significantly ($p \leq 0.05$). Different letters show statistically significant results ($p < 0.05$) of each sample during storage.

previous week was small, less than 0.1.

3.2. TPA

Hardness, adhesiveness, cohesiveness, gumminess, springiness, chewiness are the main indicators to describe the texture of yogurt. Previous studies have shown that the texture of yogurt depends on the interaction between casein micelles (Khubber et al., 2021). Fig. 2(a–f) shows the results of texture analysis of different yogurts.

Fat content is major factor in determining the hardness and adhesiveness of yogurt (Yousefi and Jafari, 2019). The hardness value of the whole yogurt (218.27 g) was significantly higher than the other five groups. With the extension of storage time, the hardness value of the six groups of yogurt increased, consistent with previous studies (Nguyen et al., 2017). In Fig. 2(b), 3.0%FFY yogurt has a higher adhesiveness than low-fat yogurts, the result revealed the effects of fat content on the adhesiveness of yogurts. Adding high concentration of TFPS reduced the adhesiveness of yogurt, which was due to the mixture of TFPS and casein forms a gel, which enhanced the strength of the structure of the gel network. Santillán-Urquiza et al. (2017) found that the addition of other

additives decreased the hardness and adhesiveness.

There was no significant difference in cohesiveness of samples during storage. Different gumminess values of different yogurts indicates that the level of fat content and the concentration of TFPS have an effect on gumminess. As shown in Fig. 2(d), the gumminess value of yogurt with 3.0% FFY was highest, and the gumminess of yogurt with TFPS was slightly higher than that of LY Control, indicating that TFPS had a positive effect on the TPA of yogurt. In terms of springiness, except for the first day, the springiness of the LY control group was weaker than other groups. For the rest of the storage time, there was no significant difference in springiness between the six groups of yogurts. With the increase of the concentration of TFPS, the springiness of yogurt slightly increased. As Xu et al. (2019) described that the springiness decreases with the increase of okra polysaccharide, the results of springiness was consistent with the results of previous studies. Chewiness represents the texture properties of yogurt, indicating its mouthfeel in the mouth. The data in Fig. 2(f) show that 3.0% FFY yogurt has the highest chewiness. In addition, the chewiness of 0.025% TLY was similar to that of 3.0% FFY. The value of chewiness was highest on the first day, decreased after a week, and remained almost unchanged for 14–21 days.

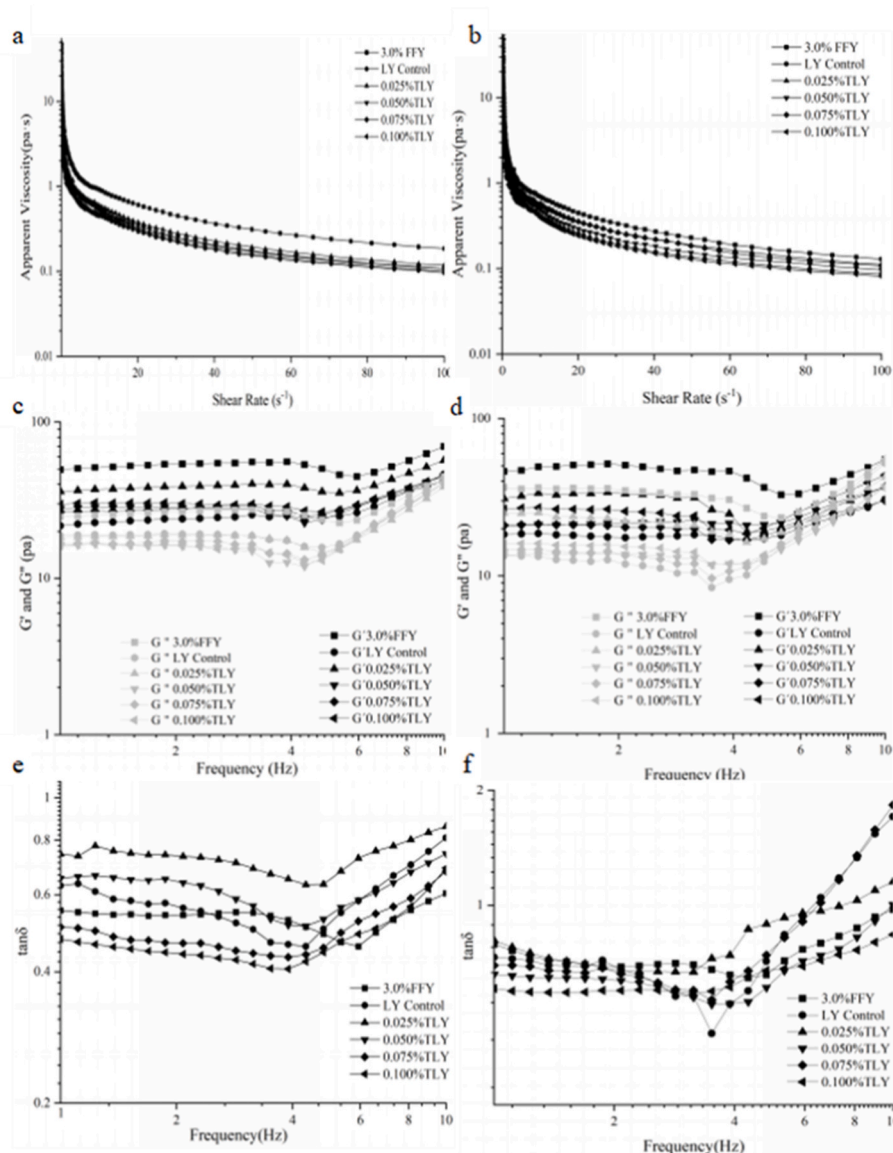


Fig. 3. The rheology of different yogurt samples stored for 21 days (a: apparent viscosity of yogurt on day 1; b: apparent viscosity on day 21; c: storage modulus and loss modulus of yogurt on day 1; d: Storage modulus and loss modulus of yogurt on day 21; e: loss tangent of yogurt on day 1; f: loss tangent of yogurt on day 21).

Generally speaking, fortification of TFPS has a positive effect on the TFPS a value of yogurt. Compared with LY Control, the TPA value of yogurt containing TFPS performs better during storage. However, the texture parameters of addition of 0.025% TFPS were the most similar with that of whole yogurt.

3.3. Rheology

The results of the apparent viscosity of all yogurt samples were shown in Fig. 3(a) and 3(b). The six groups of yogurt all showed shear thinning behavior, consistent with previous studies (Xu et al., 2019). According to Cui et al. (2014), this pseudoplastic phenomenon was caused by the breaking of protein bonds. Compared with the low-fat yogurt control, the samples containing TFPS showed a higher apparent viscosity. The increase in WHC of solids content are the most likely explanations for the phenomenon.

Frequency sweep behavior includes storage modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta$). The storage modulus represents the elasticity of the yogurt, and the loss modulus represents the viscosity of the yogurt. The loss tangent is a parameter reflecting the ratio of viscoelasticity of yogurt. As shown in Fig. 3(c) and (d), in the entire range of test frequency, the G' value of all samples was always greater than the G'' value, indicating that the yogurt system exhibits a solid-like behavior. Obviously, the G' and G'' values of yogurt added with TFPS were greater than LY Control, indicating that TFPS can improve the firmness of yogurt. A similar result was reported by Pan et al. (2019), which claimed that the interaction between the phenolic compounds of pomegranate juice powder and protein forms a stronger three-dimensional network, making the yogurt more viscoelastic. Previously, it was reported that increasing levels of guar gum also increased the complex viscosity of churned yogurt (Lee and Chang, 2016).

As negative polysaccharides, TFPS in yogurt played an important role in regulating the rheological behavior of yogurt samples (Xu et al., 2020). In the protein-anionic polysaccharide system, when the polysaccharide and the protein have opposite charges, the complex coagulation phenomenon occurs due to electrostatic attraction (Wang et al., 2020). During the continuous acidification of yogurt, the net charge of casein micelles and colloidal calcium phosphate decreases, and casein allows calcium ions to migrate inwardly, promotes interactions between proteoglycans and binds casein micelles together (Wijaya et al., 2017). As the entanglement of the polymer in the solid gel increases, the rigidity of the yogurt increases and the frequency of G' and G'' increases. Furthermore, the solid-like properties (G' and G'') were dominant in TFPS added yoghurt. Similar storage and loss modulus were found in fixed yogurt with other hydrocolloids (Xu et al., 2019). A higher G' and no crossover with G'' indicated the strong gel-like behavior of yogurt. In view of the formation of electrostatic bonds at low pH (4–5), the interaction between casein and anionic TFPS lead to the dense accumulation of protein gel structure and the filling effect of hydrated hydrocolloids. This result in the solid gel-like behavior of yogurt with stronger elasticity. Low-fat yogurt supplemented with inulin (Karimi et al., 2015) and konjac glucomannan (Dai et al., 2016) to the fat-reduced yogurt also has a higher elastic modulus.

As one can see in Fig. 3(e), all the $\tan \delta$ values of yogurts on the first day were less than 1, indicating that the storage modulus value was greater than the loss modulus, the yogurt mainly undergoes elastic deformation, and the yogurt was solid (Khubber et al., 2021). With the prolongation of storage time, the $\tan \delta$ of LY Control gradually exceeded to 1 on the 21st day, and the maximum value reached 1.83, indicating that the state of yogurt was more liquid. Compared with the control group, the yogurt containing the TFPS group had a higher storage modulus because of the higher synergistic effect of polysaccharide and protein (Chai et al., 2020). Except for LY Control and 0.075% TLY, the $\tan \delta$ value of other groups of yogurts were about 1, showing a semi-solid state. Overall, the whey precipitation of yogurt was significantly alleviated by adding TFPS, which was consistent with the result of WHC and

syneresis.

3.4. Yoghurt microstructure

Fig. 4 was a scanning electron microscope image of yogurt stored for 1 day and 21 days, showing differences in gel structure such as whey protein micelles and pore size. As observed in Fig. 4(a1), the SEM image of 3.0% FFY has a compact and uniform structure, and its microstructure was composed of casein micelles interacting with fat globules in the protein network. Compared with whole yogurt, the LY Control (Fig. 4 (b1)) shows that the structure of the gel network has larger pores, low uniformity and loose structure. Similarly, recent studies suggest that low fat content levels have an adverse effect on yogurt (Ding et al., 2022). Compared with LY Control, the yogurt with TFPS shows a finer and more uniform structure, indicating that the addition of TFPS enhance the structure of the gel network. This positive effect of TFPS on the

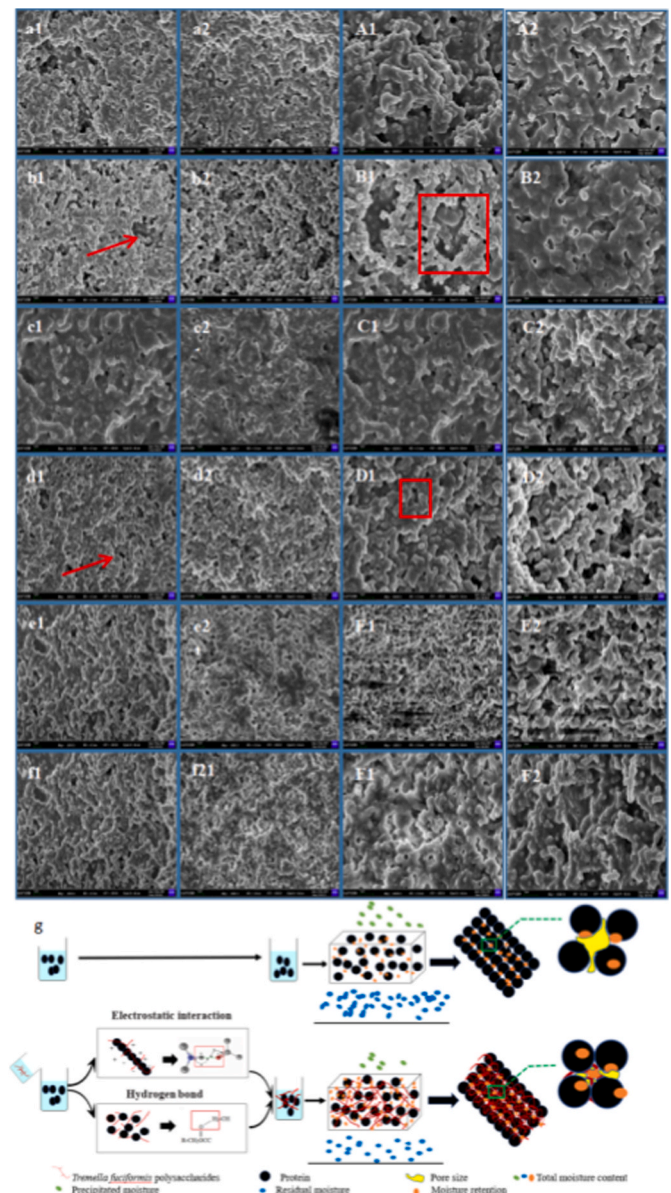


Fig. 4. (1) SEM images of different yogurt samples stored for 21 days. (A: $\times 2500$, a: $\times 10000$; A/a: 3.0% FFY; B/b: LY Control; C/c: 0.025% TLY; D/d: 0.050% TLY; E/e: 0.075 %TLY; F/f: 0.100%TLY; the number after the letter represents the storage days); (2) Schematic diagram of the effect of interaction between TFPS and protein on water retention in yogurt (g).

microstructure due to the ability of TFPS to bind water. In addition, TFPS can form insoluble gels in the form of sub-micron crystals in the water phase. When the addition amount of TFPS was 0.075%, both the pore size of microstructure and the uniformity were improved accordingly.

Over time, during the continuous acidification of milk, the casein particles attach to the clusters to form a tighter three-dimensional network structure (Xu et al., 2019). Polysaccharides are beneficial to the structural stability of yogurt, so the addition of TFPS shows a more compact structure (Parvarei et al., 2021).

Based on the above observations, a schematic diagram illustrating the interaction of TFPS and protein on water retention in yogurt was proposed, as shown in Fig. 4(g). Without TFPS, the proteins were connected by their own non-covalent bonds to form a protein network structure. After the addition of TFPS, oppositely charged casein micelles and anionic polysaccharides form complexes through electrostatic interactions between COO⁻ of anionic polysaccharides and NH₃⁺ of casein (Xu et al., 2019). The addition of TFPS was obviously beneficial to the strength and structure of the yogurt product, because TFPS has a strong viscosity and a large amount of negatively charged residues. In addition, hydrogen bonds were easily formed between polysaccharides themselves and between polysaccharides and proteins. Compared with the solution without TFPS, the solution with added TFPS made the protein

network structure tight due to the filling of polysaccharide, the pores between the networks were significantly decreased, so that the maintain the moisture of the network structure was easier to maintain, and the water separation situation was greatly improved. At the same time, it also reduces the pores between proteins, which can effectively prevent the precipitation of whey.

3.5. Sensory properties

The sensory characteristics of food are closely related to consumer preferences and determine the acceptability of the product (Janiaski et al., 2016). Appearance, texture, flavor, color, acidity and acceptability are the basic attributes of yogurt. Table 2 showed the sensory scores of different concentrations of TFPS. In relation to sensory evaluation, appearance, color, texture, and overall acceptability were significant different among groups ($p \leq 0.05$).

The score for appearance, color, texture and overall acceptability of 3.0% FFY was the highest among all samples. The sensory evaluation analysis results showed that the scores of each attribute of the yogurt were significantly improved by adding TFPS, indicating that the addition of TFPS has a positive effect on texture and taste of yogurt. Similarly, Priti et al. (2018) also found that the scores of yogurt added with gelatin were higher than those of the control group. The texture was

Table 2
Sensory evaluation of different prepared yogurts.

Parameter		Storage period (day)				Mean
		1	7	14	21	
color	3.0% FFY	8.87 ± 0.01 ^a	8.82 ± 0.01 ^a	8.79 ± 0.02 ^a	8.78 ± 0.01 ^a	8.82 ^A
	LY Control	8.81 ± 0.01 ^a	8.68 ± 0.25 ^a	8.69 ± 0.01 ^a	8.68 ± 0.01 ^a	8.72 ^B
	0.025%TLY	8.84 ± 0.01 ^a	8.79 ± 0.01 ^a	8.79 ± 0.01 ^a	8.78 ± 0.01 ^a	8.81 ^A
	0.050%TLY	8.83 ± 0.01 ^a	8.79 ± 0.01 ^a	8.78 ± 0.01 ^a	8.77 ± 0.01 ^a	8.80 ^A
	0.075%TLY	8.82 ± 0.01 ^a	8.74 ± 0.01 ^a	8.73 ± 0.01 ^a	8.72 ± 0.05 ^a	8.76 ^A
	0.100%TLY	8.82 ± 0.01 ^a	8.75 ± 0.01 ^a	8.73 ± 0.01 ^a	8.73 ± 0.01 ^a	8.76 ^A
	Mean	8.84 ^A	8.77 ^B	8.76 ^B	8.75 ^C	
texture	3.0% FFY	8.46 ± 0.07 ^a	8.22 ± 0.11 ^a	8.36 ± 0.34 ^a	8.12 ± 0.03 ^a	8.29 ^A
	LY Control	7.77 ± 0.18 ^b	7.25 ± 0.22 ^b	7.14 ± 0.04 ^c	6.83 ± 0.03 ^d	7.25 ^B
	0.025%TLY	8.49 ± 0.02 ^a	8.25 ± 0.18 ^a	8.15 ± 0.01 ^b	8.10 ± 0.01 ^{ab}	8.25 ^A
	0.050%TLY	8.46 ± 0.02 ^a	8.22 ± 0.17 ^a	8.11 ± 0.02 ^b	7.93 ± 0.02 ^{abc}	8.19 ^A
	0.075%TLY	8.44 ± 0.02 ^a	8.17 ± 0.21 ^a	8.05 ± 0.01 ^b	7.92 ± 0.04 ^{bc}	8.15 ^A
	0.100%TLY	8.40 ± 0.01 ^a	8.14 ± 0.23 ^a	8.00 ± 0.01 ^b	7.89 ± 0.01 ^{bc}	8.11 ^A
	Mean	8.34 ^A	8.04 ^B	7.97 ^B	7.80 ^C	
acidity	3.0% FFY	8.78 ± 0.07 ^a	8.65 ± 0.03 ^a	8.51 ± 0.01 ^a	8.32 ± 0.02 ^a	8.57 ^A
	LY Control	8.14 ± 0.03 ^c	8.05 ± 0.05 ^d	7.85 ± 0.11 ^d	7.51 ± 0.01 ^c	7.89 ^B
	0.025%TLY	8.73 ± 0.02 ^{ab}	8.62 ± 0.03 ^{ab}	8.50 ± 0.01 ^{ab}	8.29 ± 0.01 ^{ab}	8.54 ^A
	0.050%TLY	8.66 ± 0.01 ^{ab}	8.53 ± 0.04 ^b	8.41 ± 0.01 ^{abc}	8.23 ± 0.02 ^{ab}	8.46 ^A
	0.075%TLY	8.62 ± 0.02 ^{ab}	8.47 ± 0.03 ^{bc}	8.31 ± 0.04 ^{bcd}	8.21 ± 0.01 ^{ab}	8.40 ^A
	0.100%TLY	8.58 ± 0.01 ^b	8.42 ± 0.03 ^c	8.28 ± 0.08 ^c	8.20 ± 0.01 ^b	8.38 ^A
	Mean	8.59 ^A	8.46 ^B	8.31 ^B	8.13 ^C	
appearance	3.0% FFY	8.61 ± 0.01 ^a	8.52 ± 0.02 ^a	8.35 ± 0.01 ^a	8.23 ± 0.03 ^a	8.43 ^A
	LY Control	8.3 ± 0.02 ^c	8.26 ± 0.03 ^b	8.07 ± 0.03 ^c	7.7 ± 0.10 ^b	8.09 ^B
	0.025%TLY	8.51 ± 0.01 ^b	8.50 ± 0.01 ^a	8.31 ± 0.01 ^a	8.15 ± 0.05 ^b	8.37 ^A
	0.050%TLY	8.50 ± 0.01 ^b	8.49 ± 0.01 ^a	8.26 ± 0.01 ^a	8.08 ± 0.01 ^b	8.34 ^A
	0.075%TLY	8.49 ± 0.01 ^b	8.47 ± 0.01 ^a	8.23 ± 0.03 ^a	8.08 ± 0.03 ^b	8.32 ^A
	0.100%TLY	8.48 ± 0.02 ^b	8.45 ± 0.01 ^a	8.21 ± 0.06 ^a	8.01 ± 0.01 ^{bc}	8.29 ^A
	Mean	8.48 ^A	8.45 ^B	8.24 ^B	8.04 ^C	
flavor	3.0%FFY	8.76 ± 0.04 ^a	8.61 ± 0.04 ^a	8.44 ± 0.01 ^a	8.22 ± 0.03 ^a	8.51 ^A
	LY Control	7.86 ± 0.03 ^d	7.73 ± 0.03 ^d	7.60 ± 0.10 ^d	7.41 ± 0.03 ^d	7.65 ^B
	0.025%TLY	8.60 ± 0.01 ^b	8.50 ± 0.02 ^b	8.30 ± 0.02 ^b	8.14 ± 0.04 ^b	8.39 ^A
	0.050%TLY	8.54 ± 0.01 ^{bc}	8.49 ± 0.03 ^b	8.27 ± 0.03 ^b	8.11 ± 0.03 ^b	8.36 ^A
	0.075%TLY	8.52 ± 0.01 ^c	8.43 ± 0.02 ^{bc}	8.23 ± 0.06 ^b	8.07 ± 0.03 ^{bc}	8.32 ^A
	0.100%TLY	8.50 ± 0.01 ^c	8.41 ± 0.02 ^c	8.06 ± 0.06 ^c	8.01 ± 0.01 ^c	8.25 ^A
	Mean	8.47 ^A	8.36 ^B	8.15 ^B	8.00 ^C	
over acceptability	3.0% FFY	8.61 ± 0.01 ^a	8.41 ± 0.02 ^a	8.21 ± 0.02 ^a	8.03 ± 0.02 ^a	8.32 ^A
	LY Control	7.81 ± 0.01 ^d	7.62 ± 0.03 ^d	7.45 ± 0.03 ^d	7.16 ± 0.08 ^c	7.51 ^B
	0.025%TLY	8.56 ± 0.01 ^b	8.27 ± 0.04 ^b	8.17 ± 0.04 ^{ab}	8.02 ± 0.03 ^a	8.26 ^A
	0.050%TLY	8.50 ± 0.01 ^b	8.33 ± 0.03 ^b	8.12 ± 0.03 ^b	8.00 ± 0.01 ^a	8.24 ^A
	0.075%TLY	8.46 ± 0.01 ^b	8.25 ± 0.01 ^{bc}	8.07 ± 0.03 ^{bc}	7.97 ± 0.01 ^a	8.19 ^A
	0.100%TLY	8.38 ± 0.02 ^c	8.17 ± 0.02 ^c	8.01 ± 0.01 ^c	7.85 ± 0.05 ^b	8.10 ^A
	Mean	8.39 ^A	8.18 ^B	8.01 ^B	7.84 ^C	

^a a-e Means in the same list followed by different letters indicate significant differences ($p \leq 0.05$) between samples for the same sensory attribute.

^b A-E Means in the same row followed by different letters indicate significant differences ($p \leq 0.05$) between samples for the same sensory attribute.

consistent with the previous results of water retention and hardness, which was an important attribute characteristic to sensory quality. From the perspective of color levels, there were no significant difference in the scores of the six groups of yogurts, indicating that the addition of TFPS did not affect the color of yogurt.

The addition of TFPS without adversely affect the flavor of yogurt. In terms of overall acceptability, the score of 0.025% TLY (8.56 points) was closest to the score of 3.0% FFY (8.61 points). In low-fat yogurts, TFPS can be used as a fat substitute and provide sensory properties almost similar to those of whole yogurt. Due to the small number of sensory assessors, the scoring results may be limited. At present, the sensory score can be used as the preliminary sensory data of TFPS as a fat substitute in yogurt.

4. Conclusion

The addition of TFPS improves the physical and chemical properties, texture, rheology, microstructure and sensory properties of low-fat yogurt. Since TFPS contain a large number of negatively charged carboxyl groups and positively charged casein, as well as the water retention capacity of TFPS, the loss of whey was reduced and WHC was increased. Importantly, the addition of TFPS improved the hardness, adhesiveness, cohesiveness and gumminess of low-fat yogurt, showed more solid behavior, and played a positive role in the texture of yogurt. The study of dynamic oscillation (G' , G'' , $\tan \delta$) shows that TFPS yogurt has higher viscosity and gel strength when compared with the control group. Analysis of Microstructure also confirmed that the addition of TFPS can fill the porous protein network and enhance the three-dimensional network structure of yogurt. The addition of TFPS improves the sensory properties of symbiotic yogurt. The low-fat yogurt with 0.025% TFPS powder has the highest sensory score. In addition, TFPS are a good source of prebiotics and work synergistically with the nutritional and therapeutic properties of yogurt to produce beneficial health effects. Therefore, TFPS can be a promising fat substitute for low-fat yogurt.

CRedit authorship contribution statement

Yang Lin: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Qiaolian Xu:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Xiangmin Li:** Methodology, Writing – review & editing. **Ping Shao:** Validation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors appreciate the support by the Zhejiang province key research and development program (No. 2019C02070), State Key Laboratory of Applied Microbiology Southern China (Grant No. 2019SKLAM003).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2022.06.007>.

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